http://dx.doi.org/10.1590/1678-4162-12275

Arq. Bras. Med. Vet. Zootec., **v.73, n.5, p.1076-1084, 2021**

Effects of conditioning on the welfare of jaguars (*Panthera onca*) in captivity

[*Efeitos do condicionamento sobre o bem-estar de onças-pintadas* (*Panthera onca*) *em cativeiro*]

L.C.F. *Garcia*[1,2,3] B. *Dallago*[1,4], L.G.D. *Dantas*[3], F.E.M. *Bernal*[1]

[1]Universidade de Brasília (UnB), Faculdade de Agronomia e Medicina Veterinária, Brasília, DF, Brasil
[2]Centro Universitário do Distrito Federal (UDF), Brasília, DF, Brasil
[3]Instituto de Preservação de Felídeos da Fauna Silvestre do Brasil em Processo de Extinção (NEX), Corumbá, GO, Brasil
[4]Universidade de Brasília – UnB, Laboratório Interdisciplinar de Biociências – LabIC, Brasília, DF, Brasil

ABSTRACT

The jaguar is the largest feline in the Americas and in the face of the threat of extinction and the reduction of natural areas, keeping the species in captivity may be important for its conservation. This condition can lead to a reduction in well-being, especially due to spatial limitation and lack of environmental stimulus. In recent decades, techniques have been sought to minimize the negative impacts of captivity, with an increase in the use of environmental enrichment and operational conditioning in order to facilitate routine procedures for the animal management. In this scenario, this study aimed to evaluate the effects of conditioning on the welfare of jaguars in captivity, analyzing behavioral and physiological effects through salivary cortisol. Seven jaguars were studied in a Scientific Breeder. There was an increase in behaviors associated with welfare and cortisol during conditioning, possibly related to learning. The increase in behaviors associated with welfare suggests that the technique can contribute to improve the quality of life of these animals in captivity.

Keywords: training, feline, positive reinforcement, stress, salivary cortisol

RESUMO

A onça-pintada é o maior felino das Américas e, diante da ameaça de extinção e da redução de áreas naturais, manter a espécie em cativeiro pode ser importante para sua conservação. Essa condição pode levar à redução no bem-estar, especialmente devido à limitação espacial e à carência de estímulos ambientais. Nas últimas décadas, têm sido buscadas técnicas para minimizar os impactos negativos do cativeiro, com crescimento da utilização de enriquecimento ambiental e do condicionamento operante, com o intuito de facilitar procedimentos de rotina do manejo dos animais. Nesse cenário, o presente estudo teve por finalidade avaliar os efeitos do condicionamento sobre o bem-estar de onças-pintadas em cativeiro, analisando-se efeitos comportamentais e fisiológicos por meio do cortisol salivar. Foram estudadas sete onças-pintadas em um criadouro científico. Houve aumento dos comportamentos associados ao bem-estar e do cortisol durante o condicionamento, possivelmente relacionados à aprendizagem. O aumento nos comportamentos de bem-estar sugere que a técnica pode contribuir para melhorar a qualidade de vida desses animais em cativeiro.

Palavras-chave: treinamento, felinos, reforço positivo, estresse, cortisol salivar

INTRODUCTION

Keeping animals in captivity is an important tool for the conservation of species, as long as they are kept in conditions of health and welfare (Swaisgood, 2010). However, environments without complexity (usual captivity scenario) can lead to problems such as physiological and behavioral changes, like increase in the levels of certain steroids which are known to be associated with stress and expression of undesirable behaviors (Vasconcellos and Ades, 2012).

Corresponding author: liane.ferez@gmail.com
Submitted: December 23, 2020. Accepted: May 5, 2021.

Among the strategies to minimize the adverse effects of captivity is the use of operant conditioning, also called *positive reinforcement training* (PRT) (Laule and Desmond, 1998). This technique has been used, especially in non-human primates, which in laboratory, are trained to perform behaviors that assist in the routine (Veeder *et al.,* 2009) and veterinary procedures (Lambeth *et al*., 2006). Furthermore, when performed with appropriate techniques, by trained coaches and using positive reinforcement, conditioning can make it easier the research (Bassett *et al*., 2003), improves social interaction (Schapiro, *et al*., 2001), promotes animal welfare (Bloomsmith, *et al*., 1994) and makes the experience of zoo visitors more positive (Anderson *et al*., 2003). Obviously, these positive effects are dependent on intrinsic characteristics related to the animal, the environment and the trainer and their relationship.

Positive reinforcement training consists in reinforcing the desirable behaviors presented by the animals, being possible to reduce the stress and fear associated with certain situations, once the animals start to express choice and cooperate with the procedures, such as entering and leaving restricted areas, facilitating the performance of some clinical examinations such as auscultation, ultrasonography and collection of biological material (saliva and blood) , which allows to increase the information about the physiology of the species. Besides reducing the stress involved in some simple procedures, training can be framed as environmental enrichment, which indicates its potential as a technique for improving animal welfare rates (Laule and Desmond, 1998).

In order to know how captivity affects reproduction and animal health, methods for measuring animal stress were developed, and more significant data were obtained by studies that combined behavior analysis and physiological measures (Brown, 2006). Cortisol, frequently used in these studies, has as main adaptive function to make energy available for emergency situations, however, more functions have been demonstrated, among them, facilitate learning (Broom, 2017). To evaluate cortisol, saliva is a good option due sampling can be easily accomplished noninvasively. According to Siegford *et al*. (2003) the correlation between salivary and plasma cortisol has been described for dogs and salivary cortisol has been used in studies with domestic cats, species that is generally accepted as a research model for nondomestic cat species (Conforti *et al.,* 2011).

Studies reveal the importance of environmental enrichment for animal welfare, using the analysis of corticosteroid hormones associated with stress, collected through non-invasive methods, from feces, urine or saliva (Schwarzenberger, 2007). The effects of operant conditioning have been studied with non-human primates (Perlman *et al*., 2012), passerines (Guillette *et al*., 2015) and snakes (Emer *et al*., 2015), with no studies of felines in captivity yet recorded.

The big felines, lose habitat by deforestation and are threatened by hunting. In Brazil, the jaguar (*Panthera onca*) is the only representative of the genus. The species is on the IUCN of 2017 (Red list of Threatened Species) as "almost threatened" and with declining population (Quigley *et al*., 2017) with the greatest threats being habitat fragmentation and illegal hunting (Carvalho Jr. and Morato, 2013). In some areas the species is critically endangered, as in Caatinga and Atlantic Forest (Sumário…, 2010).

Carnivores tend to develop more abnormal behaviors in captivity, such as pacing, which consists of walking a fixed path repeatedly, usually coming and going, that may have, among its motivations, the frustration of not performing hunting behaviors, such as searching for prey and its capture (Mason *et al*., 2007). To date, a review in the literature, (that included papers, proceedings of the International Society for Applied Ethology's annual congresses, unpublished reports from 14 zoos of the Federation of Zoological Gardens of Great Britain and Ireland) recorded data on stereotyped behaviors in 8 articles, totaling 9 individuals with stereotypes (Clubb and Mason, 2007). When in captivity, it can present behavioral problems common to felines, such as reduction in exploratory behaviors, increased alert reaction, long stay at rest or in hiding, pacing, pulling one's own hair, performing suction at the tip of the tail or body members, self-mutilation (Carlstead *et al*., 1993b).

Due to the threat it suffers and the problems it may present in captivity, *Panthera onca* is the

target species of this study, which consisted in investigating the effects of operant conditioning for jaguar captive individuals, through behavioral and physiological parameters.

MATERIAL AND METHODS

The experiment was conducted at the Institute for the Preservation and Defense of Endangered Felids of Brazilian Wild Fauna (NEX), located in the city of Corumbá, Goiás, Brazil. Participated in the experiment 7 jaguars (*Panthera onca*), all in adult age, being 4 males and 3 females. The project was approved by the Ethics Committee on Animal Use of the University of Brasília (CEUA/UnB) under registration number 99746/2013.

The animals were kept in enclosures that have an average of 200m^2, which had vegetation like grass, bushes and trees, artificial lake, platforms for vertical exploration and individual holding areas, which is utilized while the animals are off exhibit during enclosure maintenance and servicing. The feeding occurred daily, in the late afternoon, with approximately 3kg of pork per individual, offered inside the indoor area.

The study consisted of four treatments, each with three repetitions on alternate days for each of the seven animals, with a seven-day interval between treatments. All treatments included behavioral observations and saliva collection. During the basal line (LB) there was no intervention in order to establish patterns of analysis (baseline data). The control treatment (CT) consisted of delivering small pieces of meat to the animals, in the amount they would receive in the conditioning phase, in order to verify if the effects observed in this phase would come from this fractional feeding or conditioning. During the conditioning treatment (CD) the animals received two training sessions each experimental day. The last treatment, after training (AT), consisted of a new observation without intervention, to evaluate the effects of the technique after its application.

The behavioral observations were performed directly, between 1:30 and 3:30 pm, by the focal animal method (Altmann, 1973) and instantaneous recording, with an interval of one minute between records, for two hours. All animals were observed simultaneously. During this period, the animals' behaviors were collected outside the session and, when the animal was being trained (about 10 minutes), it was recorded "in training" by the observers. The four observers were previously trained to identify animals and behaviors, using as basis an ethogram elaborated from preliminary observations (Tab. 1), carried out by the *ad libitum* method. The calculated agreement obtained by Kappa index showed values between observers from 0.87 to 0.97 with a maximum standard error of 0.043 and confidence interval ranging from 0.869 to 1.

The behaviors were grouped into two categories, considering the framework proposed by Carsltead *et al*. (1993b), being animal welfare indicators (AWI): all exploratory behaviors, affiliative interaction; playful behavior, roll, stretching; and stress indicators (SI): pacing and tail sucking.

Saliva collections were performed using masticators made of fire hose, as described by Montanha *et al*. (2009), who considered the quantity and quality of the material obtained satisfactory. Sampling was performed consistently and in the same order, starting at 16:30 pm, thus, the interval between meat intake and sampling was greater than 60 minutes. Actually, there was no need to offer meat before sampling as jaguars naturally chewing the fire hose. Anyway, pilot tests were performed in which saliva was collected after feeding and without any feeding, for which there was no statistical difference for the cortisol concentration. The collection procedure was carried out within the limit period of 5 minutes, between the beginning of the presentation of the hose to the animal and its collection, including time to offer and collect the fire hose. To date, for dogs, an interval of up to 4 minutes between the start of handling and collection does not affect the concentration of cortisol in the sample (Kobelt *et al.*, 2003). After the animal had chewed the material, fragments of 2cm x 4cm were collected, stored in flasks in a thermal box for subsequent centrifugation at 3000 rpm for 5 minutes. The saliva was then transferred to an identified microtube and frozen (-18 °C) until the performing analysis. The cortisol dosage was performed at the Animal Welfare Laboratory of the University of Brasilia, using commercial kits (ref. 3625-300, USA® Diagnostic) by the enzyme immunoassay method (ELISA).

Table 1. Ethogram for jaguar, elaborated and used during the study

Behavioral Category	Behavior	Definition
Inactive	Sit	Subject is resting on haunches, forelegs are braced.
	Stand	Subject is stationary in a quadrupedal position.
	Lying down	Subject is alert, with its body settled on substrate, either sternally, laterally or on back. Eyes are open.
	Asleep	Subject with its body settled on substrate, either sternally, laterally or on back. Eyes are closed.
Locomotion	Walking	Forward locomotion at a slow gait. Movement of opposite limbs, two limbs on ground.
	Running	Forward locomotion in a rapid gait, which is faster than walking, all limbs may leave ground simultaneously.
	Swimming	Subject propels itself through water using its legs.
	Climb	Subject ascends and/or descends an object or structure.
Exploratory	Sniff	Subject moves around attentively while sniffing the ground and/or objects.
	Manipulate object	Subject uses any part of body to touch, hold, move, or pick up, an object.
	Flehmen	Subject makes a grimaced facial expression, where the mouth is open, upper lip is elevated, and tongue may protrude out of the mouth.
Maintenance	Drink	Subject ingests water (or other liquids) by lapping up with the tongue.
	Eat	Subject ingests food (or other edible substances) by means of chewing with the teeth and swallowing.
	Urinate	Subject releases urine on the ground while in a squatting position.
	Defecate	Subject releases feces on the ground while in a squatting position.
	Sneeze	Involuntary reflex, characterized by a rapid exhalation of air, accompanied by a characteristic sound.
Vocalizations	Vocalization	Any emission of sound produced vocally by the animal, such as roars.
Marking	Urine spray	Subject releases a jet of urine backwards against a vertical surface or object, while standing with tail raised vertically.
	Scraping	Subject scraping on logs or other elements of the enclosure.
	Rub face	Subject touches the face, starting with the lateral region of the mouth and exerts pressure, rubbing the head over elements of the enclosure.
Training	Training	Subject participates in conditioning session.
Interaction	Agnostic interaction	Animals interact aggressively, with attacks and teeth showing.
	Affiliative interaction	Animals interact, through licking, playing, approximation.
	Reproductive interaction	Any copulation-related interaction.
	Human interaction	Subject approaches in the presence of humans, can rub its head on the enclosure grid
	Playful behavior	Subject interacts with something in a “non-serious” manner, without any agonist behaviors.
Calm	Yawn	Subject opens its mouth widely while inhaling, then closes mouth while exhaling deeply.
	Groom	Subject cleans itself by licking, scratching, biting or chewing the fur on its body. May also include the licking of a front paw and wiping it over one’s head.
	Stretching	Subject extends its forelegs while curving its back inwards.
	Roll	Subject rotates body from one side to another, while lying on the ground. During the roll, the back is rubbed against ground, the belly is exposed and all paws are in the air.
Stress indicator	Pacing	Subject walks from side to side, or repeatedly uses the same route of displacement, with no apparent goal.
	Suck / bite the tail	Subject repeatedly licks, or bites the tail region.

Each conditioning session lasted about 10 minutes. The first session started at 13:30, with the first animal. In sequence, everyone was trained and then the second session started with the same first animal, maintaining the same sequence. As primary reinforcement, 20 small pieces of meat per session were offered to the animal using a long metal tweezers (20 cm), totaling 200 grams of cattle beef per session. The clicker was used as secondary reinforcement and a stick with a rounded tip was used as a "target", that is, the target on which the animal should lean its snout to perform the behaviors (Fig. 1). Touching the target is the initial behavior for the animal to be led to the subsequent commands, directing the individual to perform the desired behaviors. The commands used were: approach; lean the muzzle against the target; follow the target; stay with the muzzle leaning against the target; stand on two hind limbs; sit.

The performance of each animal was followed with a record of the number of commands correctly performed in each of the 02 daily sessions, totaling 6 sessions for each animal. All sessions were filmed for later evaluation.

Figure 1. One of the individuals during the conditioning session. a) target used, b) clicker, c) forceps for feeding and d) camera used to register the session.

Statistical analyses were performed using SAS® software (v9.4, Cary, North Carolina) at a 5% significance level. Data were submitted to analysis of normality by Shapiro-Wilk test (PROC UNIVARIATE). Repeated measure analysis (PROC MIXED) using treatment and sex as independent variables and compound symmetry (CS) as covariance structure was performed with posterior means comparisons by Tukey test. However, there was no sex effect and an ANOVA one-way showed to be more suitable. Therefore, Kruskal-Wallis test (PROC NPAR1WAY) with treatment as independent variable was used. The variables showing

differences between treatments were submitted to Wilcoxon test in order to stablish which treatments were different each other. Correlation analysis (PROC CORR) was performed using the Spearman test and then, correlations were plotted in a heatmap using R program (R Core Team, 2021; Wei *et al.*, 2017).

RESULTS

Differences were found between treatments for activity parameters, which increased in CT and CD (P=0.0005), inactivity, which decreased in CT and CD (P=0.0009), animal welfare, which showed increased in CD, which is like to CT and AT, but different of BL. (P=0.0052) and cortisol concentration, which increased in CD and AT (P=0.0012). The average and standard deviation for cortisol concentration (µ/100 mL) and frequency of activity and well-being behaviors are presented in Tab.2.

There was no difference in behavioral parameters and cortisol concentration according to the sex of the animals.

Table 2. Cortisol concentration (µ/100mL) and frequency of the activity and welfare behaviors; mean ± standard error

Variable	Treatment								P value
	Basal Line		Control		Conditioning		After Training		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Cortisol (µg/100 mL)	0.41^{A}	0.08	0.50^{A}	0.19	1.73^{C}	1.76	0.96^{B}	0.27	0.0012
Activity Behaviors (arbitrary units)	28.47^{A}	29.70	55.00^{B}	14.99	54.33^{B}	17.49	28.00^{A}	23.05	0.0005
Inactivity Behaviors (arbitrary units)	89.95^{B}	30.84	65.23^{A}	15.18	65.42^{A}	17.33	91.14^{B}	22.36	0.0009
Stress (arbitrary units)	1.66	5.65	0.76	3.49	1.00	3.64	0.33	1.52	ns
Welfare (arbitrary units)	1.42^{A}	2.15	4.09^{AB}	3.71	6.52^{B}	4.73	3.76^{AB}	3.56	0.0052

$^{A, B, C}$ Upper case letters in the same row means significant differences.
ns = not significant.

There was a positive correlation between activity and animal welfare associated behaviors and a negative correlation between inactivity and animal welfare associated behaviors (Fig. 2).

DISCUSSION

The activity and welfare behaviors showed a positive correlation, which means that the modification caused on behavior pattern of animals by treatments is relevant to the life quality, since there was a substitution of inactivity behaviors for behaviors related to welfare, such as exploratory and non-agonistic interaction (Carsltead *et al.*, 1993b). The decrease in inactivity behaviors was also observed in studies with environmental enrichment for other felines, such as the leopard-cat (Carlstead *et al.*, 1993a), lions and tigers (Silva, 2014).

The fact that the control treatment also shows increased welfare behaviors, suggests that simply offering the food fractionated by the caretaker can improve the quality of life of the animal. This practice can improve the human-animal relationship, a concern that has been growing when it comes to the maintenance of captive animals, the welfare and safety of both (Hosey, 2008). Offering meat also showed positive effects on welfare, but only conditioning allows animals to express choices and cooperate with procedures (Lambeth, *et al.*, 2006), facilitating the work routine (Bassett *et al.*, 2003). In this way, conditioning provides greater benefits than simply offering meat to animals. The use of animal conditioning for captive animals has been widespread in zoos, bringing benefits, which include a change in the method used, with the use of positive reinforcement and intensification of the perception of the animal's body language, in order to avoid fear responses and aggressive behavior (Brando, 2012).

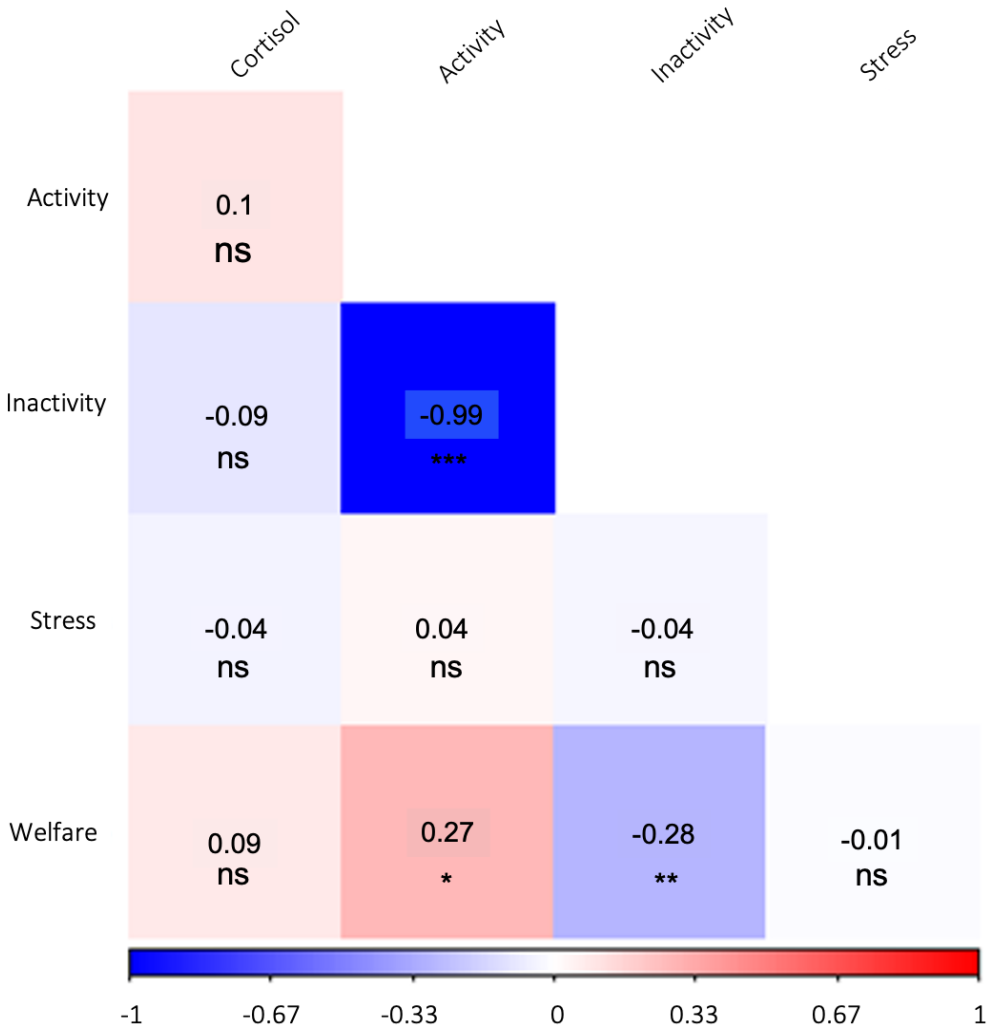

Figure 2. Results of correlation between cortisol, activity, inactivity, stress and welfare behaviors. ns = not significant, * < 0.05, ** < 0.01, *** ≤ 0.001

The increase in cortisol concentration during conditioning may be related to the involved learning during the training session. According to Broom and Zanella (2004) some studies show that cortisol facilitates learning, since receptors for this molecule were found in the amygdala and hippocampus, areas of the brain related to cognitive processes and memory. For humans, increased cortisol has been associated with enhanced memory consolidation (Cahil, *et al*., 2003). Considering that all individuals presented correct responses to the commands worked in the conditioning, it can be assumed that there was learning and perhaps the increase observed in cortisol concentration is related to this process, although this type of relationship is not yet fully understood and deserve deeper investigation.

Another possibility is that conditioning has provoked a physiological stress response, in a positive context for the animals, similar to what happens in natural events, such as hunting and reproduction (Moberg, 2000). Other studies also found an increase in glucocorticoid concentration associated with welfare behaviors, such as the experiment with environmental enrichment for pigs in which there was an increase in cortisol levels and behavioral data indicated an improvement in welfare, with a decrease in inactivity and harmful and aggressive social

behaviors (Beattie *et al*., 2000). Similar results were obtained with mice exposed to enrichment conditions, with an increase in corticosterone concentration and with behavioral parameters indicating positive effects, with an increase in "play" and socio-positive behaviors (Marashi *et al*., 2003). The increase in the concentration of glucocorticoids may represent an indicator that the stimulus was adequate, because it may be caused, for example, by the need to solve problems, resulting from conditioning, or from enrichment, thus, the interpretation of the alterations found, whether behavioral or physiological, depends on the context in which they occurred (Vasconcellos and Ades, 2012).

CONCLUSION

Training with operant conditioning can modify the behavioral and physiological responses of jaguars. The increase in cortisol may be related to the learning involved and the welfare indicator behaviors suggest that the use of this technique was positive for animal welfare.

ACKNOWLEDGEMENTS

We thank Dr. Donald Broom for his important contributions regarding the findings of the study and Cristina Gianni for making it possible to conduct the study at the Institute for the Preservation and Defense of Endangered Felids of Brazilian Wild Fauna (NEX).

REFERENCES

ALTMANN, J. Observational study of behavior: sampling methods. *Behaviour*, v.49, p.227-265, 1973.

ANDERSON, U.S.; KELLING, A.S.; PRESSLEY-KEOUGH, R. *et al*. Enhancing the zoo visitor's experience by public animal training and oral interpretation at an otter exhibit. *Environ. Behav.*, v.35, p.826-841, 2003.

BASSETT, L.; BUCHANAN-SMITH, H.M.; MCKINLEY, J.; SMITH, T.E. Effects of training on stress-related behavior of the common marmoset (*Callithrix jacchus*) in relation to coping with routine husbandry procedures. *J. Appl. Anim. Welfare Sci.*, v.6, p.209-220, 2003.

BEATTIE, V.E.; O'CONNEL, N.E.; KILPATRICK, D.J.; MOSS, B.W. Influence of environmental enrichment on welfare-related behavioural and physiological parameters in growing pigs. *Anim. Sci.*, v.70, p.443-450, 2000.

BLOOMSMITH M.A.; LAULE G.E.; ALFORD P.L.; THURSTON R.H. Using training to moderate chimpanzee aggression during feeding. *Zoo Biol.*, v.13, p.557-566, 1994.

BRANDO, A.I.C.A. Animal learning and training: implications for animal welfare. *Vet. Clin. North Am. Exotic Pract.*, v.15, p.387-398, 2012.

BROOM, D. Cortisol: often not the best indicator of stress and poor welfare. *Physiol. News*, v.107, p.30-32, 2017.

BROOM, D.M.; ZANELLA, A.J. Brain measures which tell us about animal welfare. *Anim. Welfare*, v.13, p.41-45, 2004.

BROWN, J.L. Comparative endocrinology of domestica and nondomestic felids. *Theriogenology*, v.66, p.25-36, 2006.

CAHIL L.; GORSKI, L.; LE, K. Enhanced human memory consolidation with post- learning stress: interaction with the degree of arousal at encoding. *Learn. Mem.*, v.10, p.270-274, 2003.

CARLSTEAD, K.; BROWN J.L.; STRAWN, W. Behavioral and physiological correlates of stress in laboratory cats. *Appl. Anim. Behav. Sci.*, v.38, p.143-158, 1993b.

CARLSTEAD, K.; BROWN, J.L.; SEIDENSTICKER, J. Behavioral and adrenocortical responses to environmental changes in leopard cats (*Felis bengalensis*). *Zoo Biol*., v.12, p.321-331, 1993a.

CARVALHO JR, E.A.R.; MORATO, R.G. Factors affecting big cat hunting in Brazilian protected areas. *Trop. Cons. Sci.*, v.6, p.303-310, 2013.

CLUBB, R.; MASON, G.J. Natural behavioural biology as a risk factor in carnivore welfare: how analysing species differences could help zoos improve enclosures. *Appl. Anim. Behav. Sci.*, v.102, p.303-328, 2007.

CONFORTI, V.; MORATO, R.G.; AUGUSTO, A.M. *et al*. Noninvasive monitoring of Adrenocortical function in captive jaguars (*Panthera onca*). *Zoo Biol*., v.30, p.1-16, 2011.

EMER S.A.; MORA C.V.; HARVEY M.T.; GRACE M.S. Predators in training: operant conditioning of novel behavior in wild Burmese pythons (*Python molurus bivitattus*). *Anim. Cogn.*, v.18, p.269-278, 2015.

GUILLETTE L.M.; HAHN A.H.; HOESCHELE M. *et al*. Individual differences in learning speed, performance accuracy and exploratory behaviour in black-capped chickadees. *Anim. Cogn.*, v.18, p.165-178, 2015.

HOSEY, G. A preliminary model of human–animal relationships in the zoo. *Appl. Anim. Behav. Sci.*, v.109, p.105-127, 2008.

KOBELT, A.J.; HEMSWORTH, P.H.; BARNETT, J.L. *et al*. Sources of sampling variation in saliva cortisol in dogs. *Res. Vet. Sci*., v.75, p.157-161, 2003.

LAMBETH, S.P.; HAU, J.; PERLMAN, J.E. *et al*. Positive reinforcement training affects hematologic and serum chemistry values in captive chimpanzees (*Pan troglodytes*). *Am. J. Primatol.*, v.68, p.245-256, 2006.

LAULE, G.; DESMOND T. Positive reinforcment training as an enrichment strategy. In: SHEPHERDSON, D.J.; MELLEN, J.D. HUTCHINS, M. (Orgs.). *Second nature: environmental enrichment for captive animals*. Washington: Smithsonian Institution Press, 1998. p.302-313.

MARASHI, V.; BARNEKOW, A.; OSSENDORF, E.; SACHSER, N. Effects of different forms of environmental enrichment on behavioral, endocrinological, and immunological parameters in male mice. *Horm. Behav.*, v.43, p.281-292, 2003.

MASON, G.; CLUBB, R.; LATHAM, R.; VICKERY, S. Why and how should we use environmental enrichment to tackle stereotypic behaviour? *Appl. Anim. Behav. Sci.*, v.102, p.163-188, 2007.

MOBERG, G.P. Biological response to stress: implications for animal welfare. In: MOBERG, G.P.; MENCH, J.A. (Eds.). *The biology of animal stress*. New York: CABI Publishing, 2000. p.123-146.

MONTANHA J.C.; SILVA S.L.; BOERE V. Comparison of salivary cortisol concentrations in Jaguars kept in captivity with differences in exposure to the public. *Ciênc. Rural*, v.39, p.1745-7451, 2009.

PERLMAN J.E.; BLOOMSMITH M.A.; WHITTAKER M.A. *et al.* Implementing positive reinforcement animal training programs at primate laboratories. *Appl. Anim. Behav. Sci.*, v.137, p.114-126, 2012.

QUIGLEY, H.; FOSTER, R.; PETRACCA, L. *et al*. *Panthera onca: the IUCN red list of Threatened Species*. 2017 Available in: https://www.iucnredlist.org/species/15953/123791436. Accessed in: 20 Dec. 2020.

R CORE TEAM: a language and environment for statistical computing. 2019. Available in: https://www.r-project.org/. Accessed in: 16 Apr. 2021).

SCHAPIRO S.; PERLMAN J.E.; BOUDREAU, B.A. Manipulating the affiliative interactions of group-housed rhesus macaques using positive reinforcement training techniques. *Am. J. Primatol.*, v.55, p.137-149, 2001.

SCHWARZENBERGER, F. The many uses of non-invasive faecal steroid monitoring in zoo and wild life species. *Int. Zoo Yearbook*, v.41, p.52-74, 2007.

SIEGFORD, J.M.; WALSHAW, S.O.; BRUNNER P. *et al*. Validation of a temperament test for domestic cats. *Anthrozoös*, v.16, p.332-351, 2003.

SILVA, T.B.B.; ABREU, J.B.; GODOY, A.C.; GARCIA, L.C.F. Enriquecimento ambiental para felinos em cativeiro. *Atas Saúde Ambiental*, v.2, p.44-52, 2014.

SUMÁRIO executivo do plano de ação nacional para conservação da onça pintada. Brasilia: MMA, 2010. Available in: http://www.icmbio.gov.br/portal/biodiversidade/fauna-brasileira/plano-de-acao/1344-plano-de-acao-para-conservacao-da-onca-pintada.html. Accessed in: 20 Dec. 2020.

SWAISGOOD, R.R. The conservation-welfare nexus in reintroduction programmes: a role for sensory ecology. *Anim. Welfare*, v.19, p.125-13, 2010.

VASCONCELLOS, A.S.; ADES, C. Possible limits and advances of environmental enrichment for wild animals. *Rev. Etol.*, v.11, p.37-45, 2012.

VEEDER, C.L.; BLOOMSMITH, M.A.; MCMILLAN, J.L. *et al*. Positive reinforcement training to enhance the voluntary movement of group-housed sooty Mangabeys (*Cercocebus atys atys*). *J. Am. Assoc. Lab. Anim. Sci.*, v.48, p.192-195, 2009.

WEI, T.; SIMKO, V.; LEVY, M. *et al.* R package "corrplot": visualization of a correlation matrix. 2017. Available in: ftp://xyz.lcs.mit.edu/pub/CRAN/web/packages/corrplot/corrplot.pdf. Accessed in: 16 Apr. 2021).